

Figure 1. Overlap-extension-PCR fragment with purD deletion

Overlap-extension-PCR fragment with recA deletion

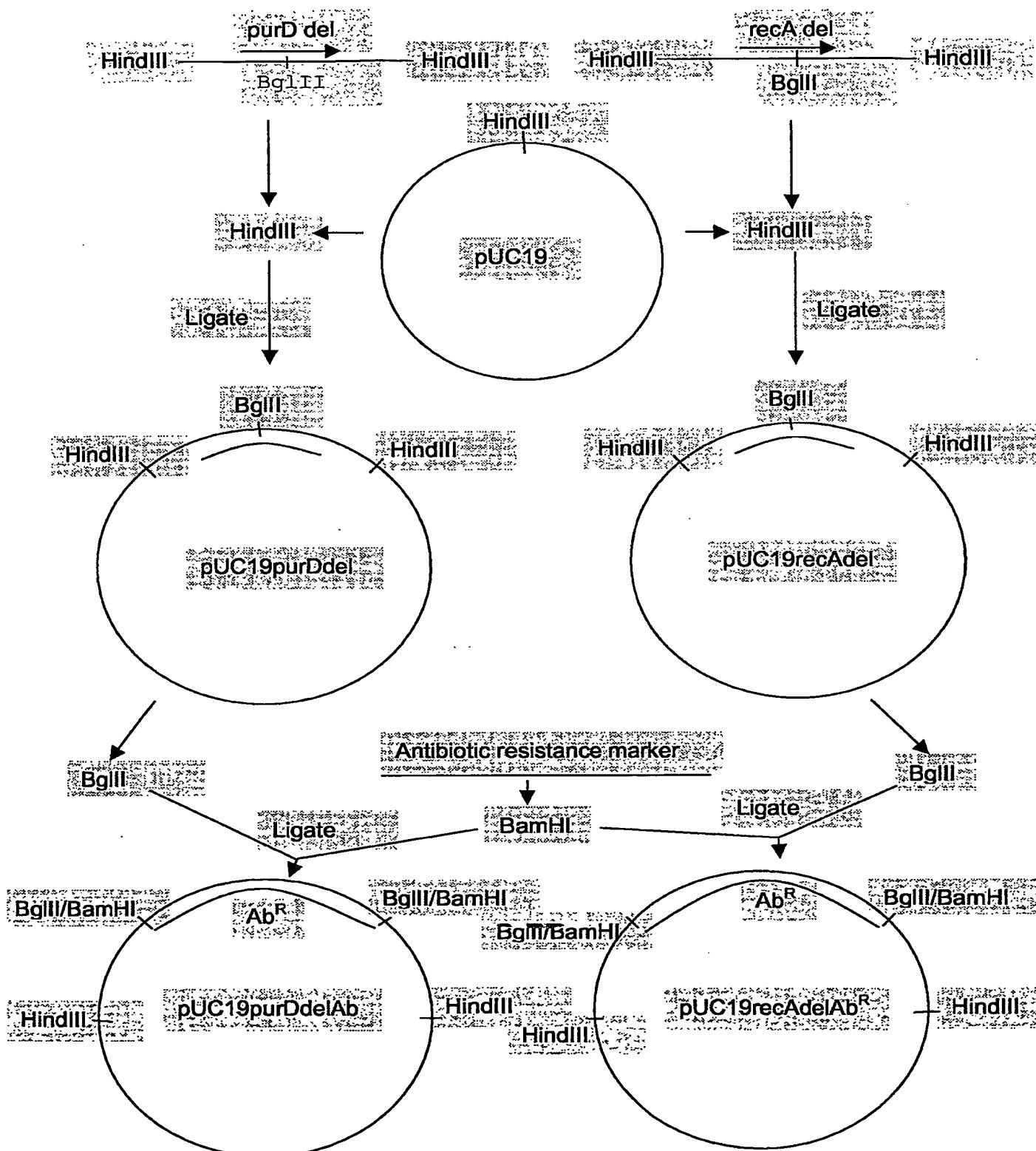


Figure 2A.

1 GTTCGACCAA ACGGCTTGT GTGCGGTGAA ACATAGCACT CCTTGTGGCG TGGCTTTAGA TGATGATATT TTGCAAGCGT
 >>.....F5.....>> CTTAAGCTTGGAA>>.....F13.....>>

HindIII

81 ACCAAAAAGC ACACGACTGC GACCCGATTT CGATTTTG TGCGATTGTA ACTTTTAATA AAAAGTAAC AAAAGCAGTG
 161 GCAGAAAAAT GTAACGAGAT TTCCCTTGAA ATCGTTGCTG CACCGAGCT TGAGCCAGAG GCTTTGGAAG TTTTGCTAA
 241 AAAGAAAAAT TTGCGCGTGA TTGAAGTTAA AAATCCATTA AGCGATAAAA TGCAACTCGT GCAAGTAGAT GGCGGATTGC
 321 TCGTGCAAGA AAACGACAAA TCGTTTAGCA ATGATTTAA AGTAGTAACC GAAAACAAC CTACCGAAAA GCAACTTTCT
 401 GATTTGGAAAT TTGCCATGAA AGTAGTGAAA CATGTAAAGA GCAATGCCAT CGTGGTTGCC ACAAACGGAC AAGCTCTAGG
 481 CGTGGGCACA GGCGAGACTA ATCGTATTG GGCAGCACAG CAGGCGATTG AGCGTGCAAA GGAAAAAACAA CAAGAAAATC
 561 TAGTTTGGC TTCCGATGCC TTTTCCCAT TCAGAGATGT GGTAGATTAT GCAGCACAAG AAGGCATTAC AGCCTTGATT
 641 CACCCAGGAG GAAGCATGCG CGACCAAGAG AGCATAGACG CGGCTAATGA ACACGGAATC CCGATGATCA TCAGCGGTAT
 721 GAGACATTTC TTACATTAAA TCAAAAAATC TAAACAATAA TTATCAATAA TTCTAAAACA CAATAAGTAT GAATGCAAAT
 >>...purD...>

801 GATTACAAA AAATACTCAT CGTAGGAAAC GGCAGCAAGAG AACACGCCAT CGGGTGGAAA ATTAAACAAG ACCACCCCTTC
 >.....>.....
 881 TTGCGAGCTT TTCTTGCGC CAGGAAACGC TGGAACCGAA CAAATTGGAA AAAACATCGT AGCTGAATCT ATTATGGCT
 >.....>.....
 <<.....OE-R.....<<AGATCTGGCGCTACGCTAGAAG

BglII

961 TAATGCTTT TGCTAACAA AATGATATAG ACTTAACGAT TGTAGGTCCA GAAGCAGAAT TGGTAGAAGG TATTGTAGAC
 >.....>.....
 1041 TTGTTGAAT CCAATCAATT AAGAATTTTT GGTCCAGATA AGCGTGGCGC TAAATTGGAA GGCGAGCAAGG CTTTTGCCAA
 >.....>.....
 1121 AGATTTATG GAGAAATACG GCGTGGCGAC GGCTTTGCC AAAAGTTCA ACAATTGT AGACGCTAGA GATTATGTAA
 >.....>.....
 1201 AAGAGCTCAC GCAATTCCCT ATCGTGTATCA AAGCCAGTGG CTTGGCAGCA GGAAAAGGTG TGATCATCGT GCACNTACAA
 >.....>.....
 1281 CTTGAAGCCG AAAACTACTTT GCGCAAATC ATGGAAGACA AAACCTTGG CGAAGCAGGC AACGAGGTG TAATCGAGGA
 >.....>.....
 1361 ATACTTAAA GGTGTGGAAG TTCTGTGCT TTCTATCTTT AACCATAAAAG AAATTTAAAC TTCTTGCCCT GTAAAAGACC
 >.....>.....
 1441 ACAAGAAAAT CGGAAAAGGC GAAACAGGAC TCAACACGGG CGGAATGGGC GTAGTGGCTC CTAACCCGCA TTTTACCGAT
 >.....>.....
 1521 GAGGCACATGA AGGAGTTGA GAAAACATT TTGCTCCCA CACAAAAAGG GCTCTGGCA GAAAAAATGC ATTGTTGCAGG
 >.....>.....
 1601 CATTATTTTC TTGGGCTTA TGATTACCGA GCATGGTATT TATCTATTGG AATACAACAT GCGATTGGC GACCCAGAAA
 >.....>.....
 1681 CCGAAGCACT TTGGCCTTG ATGGAGAATG ATTTAGTAGC CCTCATCGAT TCCGCAATAC ACCAGCAAGA CATTGAACCTT
 >.....>.....
 1761 AAATGGAAAA ACGAACATGC TTGCTGTGTA GTAATGGCGA GCGGTGGCTA CCCAGGCACT TACGAAACTG GTTTTGAAAT
 >.....>.....

1841 CCGAGGATTG AACAAAGTTG ATGTTCCGT ATTTATTGCA GGAGCCAGAG AAGAAAGTGG AAAAATCTAC ACCACAGGCG
>.....
purD.....>

1921 GGCGCGTGCT CAATGTGGTG GGAACCTGGCG CTACGCTAGA AGAACCCAGA AAAGTGGCTT ACGAAAATAT CCATAAAATC
>.....
purD.....>
GAGATCTGG>>.....OE-F.....>>

BglII

2001 AATTTGATT ATGAATATTA TCGCGAAGAC ATCGGGAAAGA TATAATCTCG CTGATTTTA ACCAAAACAT ATTTAAAAAC
>.....
purD.....>>

2081 GCTTTGTTA CTTTATAAA CAAAGGCCTT TTTCTATTT TGTCGACTA TAACATGATT TAACCCATGA AAAAAATACT

2161 AAAAAACTC ATTTTCTAC TGCTCATTCC TTGGGTTAT GCCCTGATTT TAATCTTTAT AAATCCACCT ATCACCATTA

2241 CACAGCTGAG CAATTATCT TATGGTTCT CCAGAACACA GCTCGTTAT GATGAAATTC CGGCTAGTGC TAAATGGGCT

2321 GTAATTGCAG CAGAACACCA GAATTTGCC ATTCTATAATG GCTTTGATTT TAAAGAAATT AAAACCGCCT ACGAGAAAAA

2401 CAAACGGGGC AAGAAATTGC GTGGCGGGAG CACCCCTTCG CAACAAACTG CAAAAAAATGT ATTTTGTGG CAAGGGCGCA

2481 CTTGGATTAG AAAAGGATTG GAAACCTACT GCACCTTTAT CATCGAAACG CTGTGGAGCA AGGAGCGTAT TTTGCAAGTT

2561 TACCTCAACA ATGCCGAAAT GGGCAAAGGC GTTATGGCA TAGAGGCAGC GGCAGCAATAT TATTTAAGA AAAACGCCTC

2641 ACAGCTCACG CCTACCGAGA CGGCACGCA CATTGCCCTGC CTGCCCAATC CAAAAAAATA CAANTAAAC CGGCCAAGTG

2721 CCTACATCTC AAAACGCGGA CAATGGATTG TGCGCCAAGT GCGAAACTG AAAGGCAGATA GGGCTCTGAG CGAGATTGTG

2801 AACACGCCCT AACGCCCTGCC TCAACTCTT GCACACAGTT TACCAACTCT CTGCGAAGAG TTCACAAACT CTTCGCACAC

2881 ACTTCCCCAA GTCTTGCAA AGAGTTGGGATA GATACTTAGG CACAAAAAAA AGGAACCTCA TGAATAGAGG TTCCCTCTTC

2961 CTTAAAAGGA ATAATAATA ATGTTTTTA AGCTTTAGGC TTGGCTACTT TTCAAGGC TGCTGCCCTC ATGCTATCTA

HindIII

3041 GGATACGCTT GCCTGGCGG TAGTTTACGC CTACCTTTT GATTAAGCCC GAATGAAAT CTTCTCTGT ATCTGCCGCT
<<.....R8.....<

3121 CCACTGCTTA AAGTGGCATA GAGCGAGCCA AGCTTATCTA AACGAACGAT TTGCCCCGT GCCAAGGCCTT CTTGAATTAC
<<R8.<<AAGCTTAAG

HindIII *HindIII*

3201 ATTCTCTAGC GCAATGATAA CGCCACGAAT ATCTGCCCTCG CTGAGTGGCG AAAACTCTC GATTGCTTA ACGAGCTGGT

3281 CTATATCCAT TTCTCCATCG CTTGCCACCA CGGCATAGTA TTTTGTGGC TCCCTGGCT TGCTTGGTT TCTACGCTGA

3361 ATTACATTGT ATTTTATGCT CATAATTACT CTATTTTAA TAGCCTCCCG ATGGATATAA AGTACGCTA CAATTAGGGT

3441 CTCCATAAGC AAATCTATAC CCCCTCTTT CATACTCCCT TCTCATTCTT CTGCTCCAT CTCTCAAGGC ATCCGCTCTA

3521 TTACTGCTAT ACCCCCTCCTG AAGAAATGTG TCTGCACTTG AAGAAGAATA TGAAGAGCTA TGAGAATCGT GCAACATAGT

3601 CCAAGCTCCA TCTTGAGCTA TAACATTTGC ATGACATGAA ACACCTATAG TATAATAAAA TCTCCTAGGA GGTTGTGTC

3681 CACCACCAACC TCCAGAGCTA CTACTTTTT TACATTGTCC ATTTGGTTA GCATGATTT GTCCGCCATC ACTTACTAAC

3761 TTCTTAGCTT CTGCTAAGGC TTTTCTCTT GCTTCTTT CAGCATCTGC TTGGCTAATT CCACTCACTG CTGTAGCTGT

3841 CGCTTCTTT TTATAGTTTA CCGAGGTTCC ATAATAGCCA CTACTACAAT TGTTCTGT AAAGTTTTA TAAAAAGATT

3921 GAGTTGTGT TGAGGTGTAC CCTCCGAAAC CTTTACTTC TACAGTAAAG GTAGAACTCC CCATGCTTAC GGGGAAGGTG

4001 GCGATAGTAT ACGATTGCC CTCGGCATT TGTTTACTT GATACACTCC ATCTCCTCCC ACTTCTATGC TTGCCGTTAA

4081 ATTACCACTA CCGCTAAAAG AGCCTTCTGC TATTTTAGT GTTAAATCAT TTATATCCCC TCCTGTCCCT TTTGCAGAAG

4161 CTTTTGTTAC ACTTACAGCA TCATAAGCTC CTTTCCATT GGTATAAGGT ATTTATATGG CCAAAC

Figure 2B.

1 TAAAGCTGTA AWTCGCTATA AACGCCCTT AGGATAAAAAT CTGCCATT TTGCAGTATT TTWATAGCTA AAATTTAGAA
 >>.....*FrecaOR1*.....>>

81 AACACCACATCT CGAGTAAAGG AGCGTGTAGT GCTCGCCATC GTTGAGCGAT TGCCCACCCCT CAATTGATTT GGGCGAATAC
 CTTAAGCTT>>.....*F6*.....>>

HindIII

161 TTGAAATAAA TGGCATCTTC TAGCGACACA TTTTGCGCAG AAATCATGCA AAAAGCCCCG CATAAAAAGC TGAATAAAA
 241 WGCTAWTYTT CTTGTTAAA AAAACTCATA AATTCCCCA AATATAGAAA TATTCTGTGA AAAGTTGCAA TTTATTAACA
 <<...<

321 CTATGTGCTT GCTTTTAATG AAAAAAGTAG ATTATTTTC CGAATCCGAA AGTTTATTAA CGCCCCATCC GATGCCTAGT
 <..*Freca-4*...<<

401 CCCMSCGATA GCCATGATTA ATACAAATAC AATTAAATCA WATTTTCMC MTWWACCATA GCACAACACT TGCTAGCTCA
 481 ACGAGTACTA GAGTGGTAAA AAGGATTTTG TGACGATTAT TCATGATTAA ATTTCCTCA AAGGTAATAA TTTAAACCA
 561 TAATTTCAC AATCTAAAAA TCTATTAAA TAATAGAGAA ACCAGAAAAA AATCGTATT TTACGGAATG AATAAAATGT
 641 TACAAGTAGG CGATAAAATG CCCGATTCA AAGGTGTAGA CCAATTGGG AAGGAGCATT CATCTGCCGA TTCAAAAAAT
 721 CAGAAATTAG TCGTTTTTT CTACCCAAAAA GCCAGTACGC CAGGTTGCAC GGCAGAGGCT TGCAACATCA ACGATAATCT
 801 TGATGCGCTA AAAGCACAAG GCTACCAAGT GATAGGCGTG AGTGCAGATT CGGTAGAAAA ACAACGAAAA TTCAGTGATA
 881 AATACGATT TAAATTCCCT GTGATTGCCG ATGTGGATAA GAAAATTATT GAAGCATTG GCGTGTGGGG CGAAAAGAAA
 961 TTCATGGTA AAACCTATGA CGGAATTCA CGTACGACAT TCATTATTGA TGAAAACGGA GTGGTGGAGC GCGTGATAGA
 >>.....*F7*.....>>

EcoRI

1041 AAAAGTAAA ACAAAAGATC ATACCAATCA AATTTAAAT TCAGAAAAAT AAAAATATGA GCGAAATAGA CGAAGCGAAA
 >>.....*recA*.....>

1121 AGGAAAGCAC TCCAGCTAGT GCTTGATAAA ATGGACAAAAA GCTATGGTAA AGGTGCCGTG ATGATGATGG GCGACAAAGC
 >.....*recA*.....>
 <<.....*OER1*.....<

1201 CATAGACGAA AATATTCCAG TAATCCCTAC GGGGTCTCTA GGTTTAGATT TAGCCTTGGG CGTGGGAGGG TATCCGCGCG
 >.....*recA*.....>
 <CGAGATCTCGTGCCTGCGGT

BglII

1281 GTAGAACGT GGAGATTAC GGTCCAGAAAT CTTCTGGTAA AACCACTTTG GCAATTCACTG CCATTGCCGA AGCTCAAAAG
 >.....*recA*.....>

1361 TCTGGCGAA TTGCAGCTTT CATCGATGCA GAGCACGCAT TTGATAGATA TTACGCAGAA AAATTAGGCG TAGATGTTGA
 >.....*recA*.....>

1441 GCATTTAATT ATCTCTCAGC CAGATAATGG GGAGCAAGCT TTAGAAATTG CCGATAACTT AATCCGTTCA GGTGCAATTG
 >.....*recA*.....>

HindIII

1521 ATATTATTGT AATCGATTG GTAGCGGCTT TAACGCCAAA GTCGGAAATC GACGGAGATA TGGCGATTC CAAAATGGGA
 >.....*recA*.....>

1601 TTGCAAGCGC GTTTGATGTC TCAAGCCTTG AGAAAGCTCA CGGGAACTAT CAATAAAACC AAATGTAATG CTATTTCAT
 >.....*recA*.....>

1681 CAACCAATTG AGAGAGAAAA TCGGTGTGAT GTTCGGTAGT CCAGAAACCA CAACGGGTGG TAATGCACCTT AAATTCTATG
>.....recA.....>

1761 CATCGGTGCG TCTAGACATT CGTCGTTCTA CTCAGATTAA AGATGGGAAC GATGTCATCG GAAACTTGAC TCGCGTAAAAA
>.....recA.....>

1841 GTAGTGAAAA ACAAAAGTAGC TCCGCCATTC CGTAGTGCAG AATTGACAT TATGTATGGC GAAGGAATCT CTAAGCAGG
>.....recA.....>

EcoRI

1921 CGAGATTAA GACATTGCTA CCGATTAGA AATCGTAAA AAAAGTGGCT CTTGGTATTC TTATGCAGAT ACTAAACTAG
>.....recA.....>

2001 GACAAGGGCG AGATGCCGTG CGTGCCTAT TGAAAGATAA TCCAGAATTA GCCGAAGAAT TAGAAGAGAA AATTAAAGAA
>.....recA.....>
CGAGATCT>>.....OEF1.....>>

BglII

2081 GAATTAGAGA AAAAATAGAT TTTTAGTTT TTTTAATTAA ACGAAAAATC CGTTCACTT GTGAAACGGA TTTTTTATG
>.....recA.....>>

2161 CTTGAATGAA TTTATTTCCA ATGGATTGAA TAGCCATGCA CTTTTAAATC TTCGCTATCA TAAGTGATTT CTTTGTGGT

2241 GTTGGGATAG CAAACTTAA GTCCCTGCGTA TTTGGCAATG GCATGTCCTG CGGCAATGTC CCAAAAGTTT ACAGGTCTAA

2321 AGCGGGTGTGTA CTCCGTAGCC CACCGATCGG CAATTAGCCC AAGTTGATA ACGCTTCCC TAGGCTTGT GCGGAAAATT

2401 TCATGTTCCG ATTTAATTTC TTTGATGTAT TCCTCGGTGC CAGGATCCAT GTGGAAATTG CTACAAAGAA AAGTGTAAATC

2481 TTTCGGGCAA TCCATGGTAG GAATTGGCTT GCTGTGTTTC ATCAATTGTT CAAAAAAATC CGATTTCAGA GCCATTTGT

2561 GCAATTGTTG TTGAGTCCCG ATGAATTTC GAGAAGGGCA TTTATCGCTA CCGAAATAGA ACAATCCAAG CGATGGGGCG

2641 TACAAAATC CTAGCTTAGC CGTATTATTC TCAACTAACG CTAGACACAC GCAATATTCA TCTGTTTGT TGACAAAATC

2721 CATGGTGCCA TCAATAGGGT CTGCAATCCA ATAGGTGGGC GTATTTCTAA TTTCTTGAA AGAACCTTA TCTCCTTCCT

2801 CACTAAAGTA TGGAAATGTCT GTAAAGGAAA CATGTTTTG CAAGATTGTT TTGGCGGCTA AATCTGCACT TGTAACAGGC

2881 GATCCGTGCG CTTTGGTCTC GGTGGAGAAT CCGTTTTGGA TTGTTTAAA ACCTCTCGC CAGCAAGTGC TACAGCCCCGT

2961 GTTGCATTT CTAATAAATT CATAATCATT CTTTTATTCT CGAACAAAGT CAAATAATTC TCTGTATTAA AAAATAATT

3041 TGGCGATAAA AATTAAAATT TATATATAAA ATATCTCTGC AAAAAACCAA ATCAAATATT TAGTGAATA AAAAAAATTA

3121 GATTGTAAAT TTGCCTTATG TTTTAGAGA ATACCATAAA TCATAGAAAA AATACGGCT GGATCGAAGT AATCTGTGGC

3201 TCTATGTTT CGGGCAAAAC CGAAGAGTTG ATTGTAGAG TGAAACGGAC CGAATTGGCT GGGCAAAAGG TAGAAATCTT
<<.....R5.....<<AAGCTTAAG

HindIII

3281 TAAACCCGCA ATTGATAAAC GCTACGATGA GCAAGATGTG GTATCGCATG ATGAAAACAA AAAACAAGCA ACCCCGATTG

3361 AGGCGAGTTC TAACTTGCCC ATTTAGCAA GCGATTGTGA TGTGGTGGGG ATAGATGAGG CTCAATTCTT TGACGAAGGA

3441 ATTGTTGAGG TGGCAAATCT TTTAGCTAA TCGGGAAAA GAATAATTAT TGCGGGATTA GACATGGATT TAAAGGTGCG
<<.....RrecAOR1.....<<

3521 TCCATTTGGT CCTATGCCAA ATTTAATGGC GGTAGCGGAA TATGTGACCA AAGTGCATGC AATCTGTGTG AAAACAGGGA

table 5

group	no. of chickens	Treatment			Results		
		vaccination at day 1	challenge at day 25	challenge at day 31	% of max airsec score at day 10 (safety)	% of max airsec score at day 38 (efficacy)	
1	25	NDV RecAerosol	NDV	WT-OR aerosol	2.5	25 ^b	
2	25	NDV PurD aerosol	NDV	WT-OR aerosol	7.5	23 ^b	
3	25	NDV WT-OR aerosol	NDV	WT-OR aerosol	68	10 ^b	
4	25	NDV	NDV	WT-OR aerosol	0	47	
5	25	NDV	NDV	NDV	0	2	

^bSignificantly different ($p<0.05$) compared to the controls (group 1) using two-sided Mann-Whitney U test

table 6

group	no. of chickens	vaccination		Treatment		% reduction	Results
		at day 1	day 30	challenge	day 35		
1	15	NDV	NDV	WT-OR aerosol	WT-OR aerosol	no reduction	
2	15	NDV	NDV	WT-OR aerosol	WT-OR aerosol	50% ^b	
3	15	NDV	NDV	WT-OR aerosol	WT-OR aerosol	no reduction	
4	15	MAS	MAS	WT-OR aerosol	WT-OR aerosol	no reduction	
5	15	MAS	PrD aerosol	NDV	WT-OR aerosol	50% ^b	

^bSignificantly different ($p<0.05$) compared to the controls (group 1) using two-sided Mann-Whitney U test